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Recommended Guideline for Acute Eye Irritation Testing

(U.S.) Interagency Regulatory Liaison Group
Washington, DC

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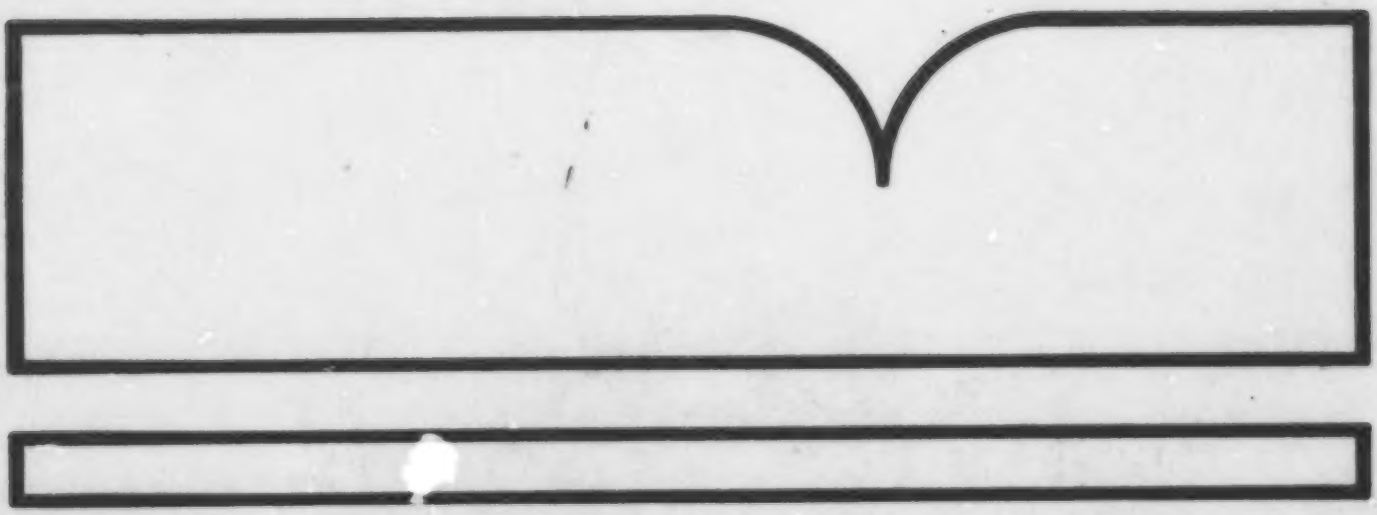

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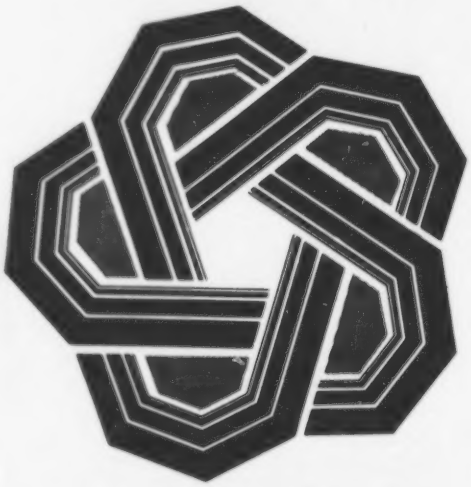
Interagency Regulatory Liaison Group

TESTING STANDARDS AND GUIDELINES WORK GROUP



Recommended Guideline For

ACUTE EYE IRRITATION TESTING

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

U.S. Consumer Product Safety Commission
U.S. Environmental Protection Agency
Food and Drug Administration (U.S. Dept. of HHS)
Food Safety and Quality Service (U.S. Dept. of Agriculture)
Occupational Safety and Health Administration (U.S. Dept. of Labor)

FOREWORD

Four regulatory agencies, the Consumer Product Safety Commission (**CPSC**), the Environmental Protection Agency (**EPA**), the Food and Drug Administration (**FDA**), and the Occupational Safety and Health Administration (**OSHA**), agreed to work together to reform the regulatory process and to improve protection of workers, public health, and the environment (**42 FR 54856, 11 October 1977**). They formed the Interagency Regulatory Liaison Group (**IRLG**) to implement their agreement. In January 1979, the Food Safety and Quality Service (**FSQS**), Department of Agriculture, joined the IRLG.

These agencies recognized that although they often regulate the same chemicals, toxicity testing guidelines used by each agency are not always uniform. Among currently required tests, differences exist primarily in details of methodology and not in fundamental toxicological principles. The Testing Standards and Guidelines Work Group was established for the purpose of developing guidelines which would resolve existing differences and be used by all of the IRLG agencies for testing chemicals for health or environmental effects or both. This effort was to be coordinated with the development of other guidelines. On December 17, 1977, the Work Group held a public meeting to explain its purpose and goals, and to answer questions about its activities (**42 FR 59106, 15 November 1977**).

IRLG guidelines are derived from those currently used or under development in the agencies, in international organizations, and in industry. Early drafts were circulated through each agency for review, and comments from that review are included in this guideline. In addition, drafts of the EPA Office of Pesticides Programs (**OPP**) proposed guidelines (**43 FR 37336, 22 August 1978**) under the Federal Insecticide, Fungicide and Rodenticide Act (**FIFRA**), and public comments to those guidelines were used extensively in an effort to assure compatibility and to benefit from the appreciable time and effort the OPP staff put into their development. A second major source of information was *Principles and Procedures for Evaluating the Toxicity of Household Substances* (**NAS Pub. No. 1138**), prepared for CPSC by the National Academy of Sciences. Still others heavily relied on were the Pharmaceutical Manufacturers' Association *Guidelines for the Assessment of Drug and Medical Device Safety in Animals* (**February 1977**); the FDA, Bureau of Foods Direct Additive Cyclic Review Draft Guidelines; the *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*, Association of Food and Drug Officials of the U. S.; and protocols submitted by several industrial organizations in response to **43 FR 1987, 13 January 78**. The Work Group also used written and verbal comments received from the public in response to **44 FR 49015, 21 August 1979**. IRLG guidelines are being coordinated with development of guidelines by the Organization for Economic Cooperation and Development (**OECD**). The Work Group acknowledges the contributions from each of these information sources and takes this opportunity to express its appreciation to the scientists who developed them and made them available.

Under most circumstances, the data obtained from this test should be sufficient to meet the requirements for a specific aspect of the toxicological characterization of the test substance. Under certain circumstances, however, the data may show a need for further study; or it may be recognized at the outset this guideline may have to be modified by an agency. It is important that tests be agreed upon between the investigator and the agency requiring them.

Each agency has the responsibility to decide what substance, and what form of the substance, it requires to be tested; which tests it will require; and how it will use the data derived from the tests.

INTRODUCTION

This guideline delineates test procedures to evaluate the toxicity of liquids, solids, aerosols, and liquids propelled under pressure, to ocular tissues of laboratory animals. The test should demonstrate the potential of a substance to produce injury to the human eye.

For humane reasons, substances known to be corrosive may be assumed to be eye irritants and should not be tested in the eye. Furthermore, substances shown to be severe irritants in dermal toxicity tests may be assumed to be eye irritants and need not be tested in the eye.

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I. General Considerations

A. Good Laboratory Practices

Studies should be conducted according to good laboratory practice regulations (*e.g.*, "Nonclinical Laboratory Studies, Good Laboratory Practice Regulations," 43 FR 59986, 22 December 1978).

B. Test Substance

1. The specific substance or mixture of substances to be tested should be determined in consultation with the responsible agency. As far as is practical, composition of the test substance should be known. Information should include the name and quantities of all major components, known contaminants and impurities, and the percentage of unknown materials to account for 100% of the test sample.

2. Ideally, the lot of the substance tested should be the same throughout the study. The test sample should be stored under conditions that maintain its stability, strength, quality, and purity from the date of its production until the tests are complete.

C. Animals

1. Healthy animals, without eye defects or irritation and not subjected to any previous experimental procedures, must be used.

2. The test animal shall be characterized as to species, strain, sex, weight and/or age. Each animal must be assigned an appropriate identification number.

3. Recommendations contained in DHEW pub. no (NIH) 74-23, entitled *Guide for the Care and Use of Laboratory Animals*, should be followed for the care, maintenance, and housing of animals.

4. Animals should be individually caged for this test.

5. Animals must be observed as necessary to insure that none is lost due to management problems.

D. Documentation

Color photographic documentation may be used to verify gross and microscopic findings. If photographs are taken, the equipment and film must be of sufficient quality to permit controlled, close-up color photography of the eye to yield clear, sharp-focus images that literally fill the camera field.

II. Specific Considerations

A. Test Preparation

1. Testing should be performed on young, adult, albino rabbits (male or female) weighing about 2.0 - 3.0 kilograms. Other species may also be tested for comparative purposes.

2. For a valid eye irritation test, at least 6 rabbits must survive the test for each test substance.

3. Animal facilities should be designed and maintained to exclude intense and direct light, sawdust, wood chips, or other extraneous materials that might produce eye irritation.

4. A trial test on 3 rabbits is suggested. If the substance produces corrosion, severe irritation, or no irritation, then no further testing is necessary. However, if equivocal responses occur, testing in at least 3 additional animals should be performed. If the test substance is intended for use in or around the eye, then testing on at least 6 animals should be performed.

B. Test Procedure

1. Both eyes of each animal in the test groups must be examined using optical instruments, fluorescein, ultraviolet light, or other appropriate means within 24 hours before substance administration.

2. For most purposes, anesthetics should not be used; however, if the test substance is likely to cause extreme pain, local anesthetics may be used prior to instillation of the test substance for humane reasons. In such cases, anesthetics should be used only once, just prior to instillation of the test substance; the eye used as the control in each rabbit should also be anesthetized.

   For substance administration, the animal is held firmly but gently until quiet. The test substance is placed in one eye of each animal by gently pulling the lower lid away from the eyeball (conjunctival cul-de-sac) to form a cup into which the test substance is dropped. The lids are then gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. Vehicle controls are not included. If a vehicle is suspected of causing irritation, additional studies should be conducted, using the vehicle as the test substance.

3. For testing liquids, 0.1 milliliter is used. For solid, paste, particulate substances (flake, granule, powder, or other particulate form), the amount used must have a volume of 0.1 milliliter weighing not more than 100 mg. The volume measurement should be taken after gently compacting the particulates by tapping the measuring container in a way that will *not* alter their individual form. The weight of the 0.1 milliliter test dose must be recorded.

4. For aerosol products, the eye should be held open and the substance administered in a single, short burst for about one second at a distance of about 4 inches directly in front of the eye. The velocity of the ejected material should not traumatize the eye. The dose should be approximated by weighing the aerosol can before and after each treatment. For other liquids propelled under pressure, such as sub-

stances delivered by pump sprays, an aliquot of 0.1 ml should be collected and instilled in the eye as for liquids.

After the 24-hour examination, the eyes may be washed, if desired. Tap water or isotonic solution of sodium chloride (U.S.P. or equivalent) should be used for all washings.

C. Observations

1. The eyes should be examined at 24, 48, and 72 hours after treatment. At the option of the investigator, the eyes may also be examined at 1 hour and at 7, 14 and 21 days. In addition to the required observations of the cornea, iris, and conjunctivae, serious lesions such as pannus, phlyctena, and rupture of the globe should be reported. The grades of ocular reaction (Table I) must be recorded at each examination.

2. After the recording of observations at 24 hours, the eyes of any or all rabbits may be further examined after applying fluorescein stain. For this optional examination, one drop of fluorescein sodium ophthalmic solution (U.S.P. or equivalent) is dropped directly on the cornea. After flushing out the excess fluorescein with tap water or isotonic solution of sodium chloride (U.S.P. or equivalent), injured areas of the cornea appear yellow and are best seen under ultraviolet illumination.

3. A record of the discharge from treated eyes is not required; however, any exudate above normal may be recorded as additional information.

4. An animal has exhibited a positive reaction if the test substance has produced at any observation one or more of the following signs:

   (a) ulceration of the cornea (other than a fine stippling)

   (b) opacity of the cornea (other than a slight dulling of the normal luster),

   (c) inflammation of the iris (other than a slight deepening of the rugae or a light hyperemia of the circumcorneal blood vessels), or

   (d) an obvious swelling in the conjunctivae (excluding the cornea and iris) with partial eversion of the eyelids or a diffuse crimson color with individual vessels not easily discernible.

D. Evaluation

1. The test result is considered positive if four or more animals in either test group exhibit a positive reaction. If only one animal exhibits a positive reaction, the test result is regarded as negative. If two or three animals exhibit a positive reaction, the investigator may designate the substance to be an irritant. When two or three animals exhibit a positive reaction and the investigator does not designate the substance to be an irritant, the test shall be repeated using a different group of six animals. The second test result is considered positive if three or more of the animals exhibit a positive reaction. Opacity grades 2 to 4 and/or perforation of the cornea are considered to be corrosive effects when opacities persist to 21 days.

2. If only one or two animals in the second test exhibit a positive reaction, the test should be repeated with a different group of six animals. When a third test is needed, the substance will be regarded as an irritant if any animal exhibits a positive response.

TABLE I

Grades for Ocular Lesions

CORNEA

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4

IRIS

Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

CONJUNCTIVAE

Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2*
Diffuse beefy red	3

Chemosis: lids and/or nictitating membranes

No swelling	0
Any swelling above normal (includes nictitating membranes)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

* Readings at these numerical values or greater indicate positive responses.

III. Data Reporting

A. Identification

Each test report should be signed by the persons responsible for the test and identify:

1. The laboratory where the test was performed by name and address;

2. The inclusive dates of the test; and

3. Each person primarily responsible for separate components of the test including (a) the conduct of the test, (b) analysis of the data, (c) the writing of the report, and (d) any written or other matter contained in the report.

B. Body of Report

The test report must include all information necessary to provide a complete and accurate description and evaluation of the test procedures and results in the following sections:

1. Summary and conclusions: This section of the test report should contain a brief description of the methods, a summary of the data, an analysis of the data, and a statement of the conclusions. The summary must highlight all positive data and observations and any other indications of toxic effects.

2. Materials: This section of the test report should include, but not be limited to, the following information:

   (a) Identification of the test substance, so far as practical, including:

      i. chemical name, molecular structure, and a qualitative and quantitative determination of its chemical composition, including names and quantities of known contaminants and impurities and listing the percentage of unidentifiable materials to account for 100% of the test sample.

      ii. manufacturer and lot number of the substance tested, and such information as physical state, pH, stability, and purity; and

      iii. specific identification of diluents, suspending agents, emulsifiers, or other materials used in administering the test substance.

   (b) Animal data, including:

      i. species and strain used and rationale for selection of the strain if other than a common laboratory strain;

      ii. source of supply of the animals;

      iii. description of any pre-test conditioning;

      iv. numbers, age and condition of animals of each sex in each test group.

(c) Data on husbandry should include description of the caging conditions including bedding material, diet, ambient temperature, humidity, and lighting conditions.

3. Methods:

(a) Deviation from guidelines—This section shall indicate all ways in which the test procedure deviates from this guideline and shall state the rationale for such deviation.

(b) Specification of test methods—This section shall include a full description of the experimental design and procedure, the length of the study, and the dates on which the study began and ended.

(c) Data on dosage administration, including:

i. all dose levels administered;

ii. method, frequency, duration, time of day; and

iii. total volume of substance (*i.e.*, test substance plus vehicle) contained in individual dosages.

(d) Data on observation methods, including:

i. duration; and

ii. method and frequency of observation of the animals.

4. Results:

The tabulation of data and individual results must accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show the relationship of effects to time of dosing, etc.

IV. Suggested Reading

1. Draize, J. H., 1959. Dermal Toxicity. *In*: Association of Food and Drug Officials of the U.S. Austin, Texas. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. pp. 46-59.

2. Green, W. R., J. B. Sullivan, R. M. Hehir, L. F. Scharpf and A. W. Dickinson, 1978. A systematic comparison of chemically-induced eye injury in the albino rabbit and rhesus monkey. The Soap and Detergent Association, New York.

3. National Academy of Sciences—National Research Council, 1977. *In*: Principles and Procedures for Evaluating the Toxicity of Household Substances prepared by the Committee for the Revision of NAS Publication 1138 for the Consumer Product Safety Commission. Eye Irritation, pp. 41-54.

4. Prince, J. H., 1964. The Rabbit in Eye Research. Springfield, Ill. Charles C. Thomas.

TESTING STANDARDS AND GUIDELINES WORK GROUP
INTERAGENCY REGULATORY LIAISON GROUP

Chairman:

Dr. Victor Morgenroth, III	Food and Drug Administration

Surrogate Liaison:

Dr. Allen H. Heim	Food and Drug Administration

Member:

Dr. James R. Beall	Department of Energy
Dr. William D'Aguanno	Food and Drug Administration
Dr. Charles Grieshaber	National Cancer Institute (NTP)
Dr. Stanley Gross	Environmental Protection Agency
Dr. Zdenka Horakova	Food Safety & Quality Service
Dr. Patricia Marlow	Occupational Safety & Health Administration
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Dr. Robert Osterberg	Food and Drug Administration
Dr. Norbert Page	Environmental Protection Agency
Dr. Orville Paynter	Department of Commerce
Ms. Peggy Perry	Food and Drug Administration
Dr. John Quest	Food and Drug Administration
Mr. Van Seabaugh	Consumer Product Safety Commission
Dr. Frode Ulvedal	Environmental Protection Agency

The IRLG Testing Standards and Guidelines Work Group dedicates its guidelines to the memory of Dr. William D'Aguanno, whose commitment and talent contributed immeasurably to their development.